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(71) Applicant: FORBES MEDI-TECH INC. [CA/CA]; 200-750 West Pender Street, Vancouver, British Columbia V6C 2T8 (CA).

(72) Inventors: KUTNEY, James, P.; 2118 Nanton Avenue, Vancouver, British Columbia V6L 3C7 (CA). MILANOVA, Radka, K.; 605-1749 Robson Street, Vancouver, British Columbia V6G 1E1 (CA). CHEN, Honming; 216-2730 Acadia Road, Vancouver, British Columbia V6T 1R9 (CA). STOYNOV, Nikolay, Mintchev; 308-1749 Robson Street, Vancouver, British Columbia V6G 1E1 (CA).

(74) Agent: BEN-OLIEL, Susan, M., M.; 2983 West 41st Avenue, Vancouver, British Columbia V6N 3C8 (CA).

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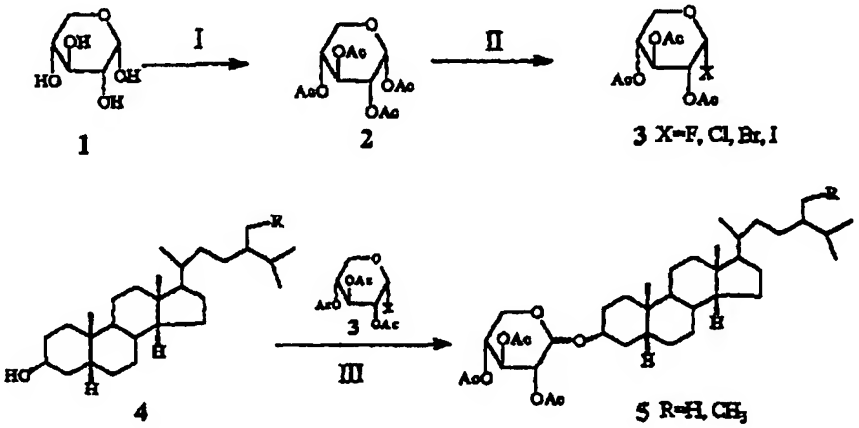
(54) Title: NOVEL GLYCOSIDES COMPRISING PENTOSE MONO-, DI-, TRI-, OR OLIGOSACCHARIDES AND PHYTOSTEROLS AND/OR PHYTOSTANOLS



I. Ac_2O, Pyridine;
II. HX (X=F, Br, Cl, I), 0-5°C;
III. $Hg(CN)_2$, $ClCH_2CH_2Cl$;

(57) Abstract: A novel glycoside compound for use in treating or preventing cardiovascular disease and it's underlying conditions such as atherosclersis and hypercholeterolemia comprises a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccharides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof.

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NOVEL GLYCOSIDES COMPRISING PENTOSE MONO-, DI-, TRI-, OR OLIGO-SACCHARDES AND PHYTOSTEROLS AND/OR PHYTOSTANOLS

FIELD OF THE INVENTION

This present invention relates to the field of phytosterols and phytostanols and their use in treating and preventing cardiovascular disease and other disorders.

BACKGROUND OF THE INVENTION

While recent advances in science and technology are helping to improve quality and add years to human life, the prevention of atherosclerosis, the underlying cause of cardiovascular disease ("CVD") has not been sufficiently addressed. Atherosclerosis is a degenerative process resulting from an interplay of inherited (genetic) factors and environmental factors such as diet and lifestyle. Research to date suggest that cholesterol may play a role in atherosclerosis by forming atherosclerotic plaques in blood vessels, ultimately cutting off blood supply to the heart muscle or alternatively to the brain or limbs, depending on the location of the plaque in the arterial tree (1,2). Overviews have indicated that a 1% reduction in a person's total serum cholesterol yields a 2% reduction in risk of a coronary artery event (3). Statistically, a 10% decrease in average serum cholesterol (e.g. from 6.0 mmol/L to 5.3 mmol/L) may result in the prevention of 100,000 deaths in the United States annually (4).

Sterols are naturally occurring compounds that perform many critical cellular functions. Phytosterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. The dietary source of phytosterols in humans comes from plant materials i.e. vegetables and plant oils. The estimated daily phytosterol

content in the conventional western-type diet is approximately 60-80 milligrams in contrast to a vegetarian diet which would provide about 500 milligrams per day.

Phytosterols have received a great deal of attention due to their ability to decrease serum cholesterol levels when fed to a number of mammalian species, including humans. While the precise mechanism of action remains largely unknown, the relationship between cholesterol and phytosterols is apparently due in part to the similarities between the respective chemical structures (the differences occurring in the side chains of the molecules). It is assumed that phytosterols displace cholesterol from the micellar phase and thereby reduce its absorption or possibly compete with receptor and/or carrier sites in the cholesterol absorption process.

Over forty years ago, Eli Lilly marketed a sterol preparation from tall oil and later from soybean oil called Cytellin™ which was found to lower serum cholesterol by about 9% according to one report (5). Various subsequent researchers have explored the effects of sitosterol preparations on plasma lipid and lipoprotein concentrations (6) and the effects of sitosterol and campesterol from soybean and tall oil sources on serum cholesterols (7). A composition of phytosterols which has been found to be highly effective in lowering serum cholesterol is disclosed in US Patent Serial No. 5,770,749 to Kutney et al. and comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol (beta-sitostanol). It is noted in this patent that there is some form of synergy between the constituent phytosterols, affording even better cholesterol-lowering results than had been previously achieved.

Despite the obvious and now well recorded advantages of phytosterols, not only in the treatment of CVD and its underlying conditions such as hypercholesterolemia, hyperlipidemia, atherosclerosis, hypertension, thrombosis but in the treatment of other diseases such as Type II diabetes, dementia cancer and aging, the administration of phytosterols and the incorporation thereof into foods, pharmaceuticals and other delivery

vehicles has been complicated by the fact that they are highly hydrophobic (i.e.they have poor water solubility). The provision of a water-soluble phytosterol derivative which could be administered orally and which could be incorporated without further modification into delivery vehicles would be highly desirable and has not heretofore been achieved.

It is an object of the present invention to obviate or mitigate the above disadvantages.

SUMMARY OF THE INVENTION

The present invention provides novel glycosides comprising a carbohydrate moiety and a non-carbohydrate moiety, wherein the carbohydrate moiety is selected from the group consisting of pentose mono-, di-, tri- or oligo- saccharides and their acylated derivatives and the non-carbohydrate moiety is a phytosterol or phytostanol, or derivative thereof.

The present invention further comprises a composition for treating or preventing CVD and its underlying conditions including atherosclerosis, hypercholesterolemia, hyperlipidemia, hypertension, thrombosis, and related diseases such as Type II diabetes, as well as other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer which comprises one or more novel glycosides comprising a carbohydrate moiety and a non-carbohydrate moiety, wherein the carbohydrate moiety is selected from the group consisting of pentose mon-, di-, tri- or oligo- saccharides and their acylated derivatives and the non-carbohydrate moiety is a phytosterol or phytostanol, or derivative thereof and a pharmaceutically acceptable carrier or adjuvant therefor.

The present invention further provides foods, beverages and nutraceuticals supplemented with one or more of the pentose-based glycosides described herein.

The present invention further provides a method for treating or preventing CVD and its underlying conditions including atherosclerosis, hypercholesterolemia, hyperlipidemia, hypertension, thrombosis, and related diseases such as Type II diabetes, as well as other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer by administering to an animal one or more of the pentose-based glycosides comprising phytosterols and/or phytostanols as described herein.

The present invention further provides a process of preparing glycosides comprising a carbohydrate moiety and a non-carbohydrate moiety, which comprises:
a) protecting the carbohydrate moiety;
b) converting the protected carbohydrate moiety to a halide or halide/acetate derivative; and then
(c) adding phytosterol or phytostanol to the derivative under reaction conditions suitable to form a glycoside.

The pentose-based glycosides comprising phytosterol and/or phytostanol of the present invention have numerous advantages over non-modified phytosterol/stanol compositions previously known and described in the art. In particular, it has been found that solubility in aqueous solutions such as water is improved thereby allowing oral administration per se without any further enhancements or modifications. Accordingly, the derivatives of the present invention can be prepared and used as such or they can be easily incorporated into foods, beverages, pharmaceuticals and nutraceuticals regardless of whether these "vehicles" are water-based. This translates into an economic advantage when preparing phytosterols and/or phytostanols for incorporation into these consumer products.

BRIEF DESCRIPTION OF THE DRAWING

The present invention is illustrated by the following non-limiting drawings in which:

Figure 1 is a flow chart showing one preferred process of preparing a derivative of the present invention comprising a pentose mono-, di-, tri- or oligosaccharide bonded to a phytosterol or phytostanol through a glycosidic linkage;

Figure 2 is a flow chart showing another preferred process of preparing a derivative of the present invention comprising a pentose mono-, di-, tri- or oligosaccharide bonded to a phytosterol or phytostanol through a glycosidic linkage; and

Figure 3 is a flow chart showing a process of "freeing" the protected groups of the carbohydrate moiety.

PREFERRED EMBODIMENTS OF THE INVENTION

According to an embodiment the present invention, there are provided one or more novel phytosterol and/or phytostanol glycoside derivatives suitable for use per se in treating or preventing CVD and its underlying conditions, such as atherosclerosis, hypercholesterolemia, hyperlipidemia, hypertension, thrombosis, and related diseases such as Type II diabetes, as well as in treating and preventing other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer. The derivatives of the present invention comprise a pentose mono-, di-, tri- or oligosaccharide bonded to a phytosterol or phytostanol through a glycosidic linkage.

Phytosterols/Phytostanols

The non-carbohydrate moiety of the novel glycoside of the present invention is defined herein as a phytosterol, a phytostanol or a derivative of either. For greater clarification, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include modified side chains also falls within the purview of this invention. It is also to be understood that, when in doubt throughout the specification, the term "phytosterol" encompasses both phytosterol and phytostanol i.e. the terms may be used interchangeably unless otherwise specified.

The phytosterols and phytostanols for use in forming the glycoside derivatives in accordance with this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sesame oil and fish oils. Without limiting the generality of the foregoing, it is to be understood that there are other sources of phytosterols and phytostanols such as marine animals from which the composition of the present invention may be prepared. US Patent Serial No. 4,420,427 teaches the preparation of sterols from vegetable oil sludge using solvents such as methanol. Alternatively, phytosterols and phytostanols may be obtained from tall oil pitch or soap, by-products of forestry practises as described in US Patent Serial No.5,770,749, incorporated herein by reference.

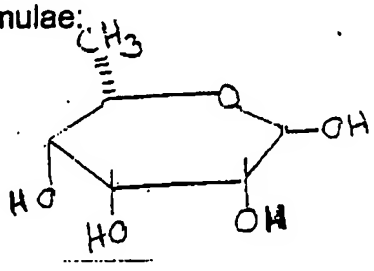
In a most preferred form, the derivative of the present invention is formed with naturally-derived or synthesized sitostanol or with naturally derived or synthesized campestanol or mixtures thereof.

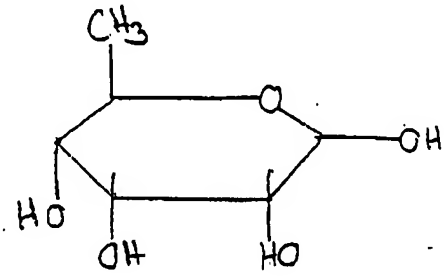
Pentoses

The carbohydrate moiety of the novel glycoside of the present invention is defined herein as a pentose mono-, di-, tri- or oligo- saccharide or one of its acylated derivatives. For greater clarity, the term "pentose" means a carbohydrate having five carbon atoms and encompasses aldehyde-containing pentoses (aldopentoses), ketone-containing pentoses (ketopentoses) and all acylated derivatives thereof including methyl pentoses.

For example, the following eight aldopentoses, including shown stereoisomers, are within the purview of the present invention: D-ribose, D-arabinose, D-xylose, D-lyxose, L- ribose, L-arabinose, L-xylose and L-lyxose. Aldopentoses may also occur in an alpha or beta anomeric configuration depending on the position of the C-1 hydroxyl group. Further, ketopentoses such as D-ribulose, D-xylulose, L-ribulose and L-xylulose are also suitable for forming the glycoside derivatives as described herein.

In methyl pentoses, one of the hydrogen groups of the primary alcohol is replaced by methyl. Examples of methyl pentoses include L-rhamnose, L-isorhamnose, D-isorhamnose, fucose, rhodeose, epirhodeose and two methyl pentoses having the following structural formulae:





Any pentose mono-, di-, tri- or oligosaccharide (polysaccharide) can be used to form the glycoside derivatives of the present invention. Most preferred, however, are the two pentoses L-arabinose and D-xylose due to their wide and ready availability. Preferred disaccharides include sucrose, maltose and lactose. Preferred oligosaccharides include starch. The two above noted pentoses, L-arabinose and D-xylose are widely distributed

in vegetables as polysaccharides of high molecular weight, and are also called "pentosans". Xylose is found in oat hulls and arabinose in gums, the latter may be conveniently prepared from cherry gum or gum arabic. The D-isomer of arabinose can be synthetically prepared from D-glucose by various degradation methods known in the art.

Glycoside Derivative Formation

Glycosides compounds comprising a sugar portion linked to a non-sugar moiety (also called the "aglycone") via a particular linkage called a "gylcosidic" linkage. In the vast majority of glycosides, this linkage is a hemi-acetal linkage formed by the reducing group of the sugar (usually an aldehyde or keto group) and an alcoholic or phenolic hydroxyl group of the aglycone. In the sugar moiety, usually, but not always, the carbon atom that is linked to the two oxygen atoms (for example C-1 in pentose) is the one forming this glycosidic linkage, in which it is linked, through an oxygen bridge, to the carbon (in the aglycone) carrying the alcoholic or phenolic group.

Within the scope of the present invention, the aglycones are phytosterols, phytostanols or derivatives thereof and the sugar or carbohydrate moiety is derived from pentose, thereby forming plant sterol-based "pentosides". It is to be understood that, in the pentose forming the gylcoside, the configuration at the carbon atom forming the linkage may be in the alpha or beta form (one of the two diastereoisomers) giving rise to an alpha-glycoside or a beta-glycoside respectively.

The pentosides of the present invention may comprise only one molecule of a monosaccharide (for example, xylose or arabinose) or in an alternative form, may comprise two or more monosaccharide units linked together in a disaccharide, trisaccharide or oligo(poly)saccharide fashion. Most often, these saccharide chains are then linked to the phytosterol or phytostanol at one position, rather than having two or

three sugars linked to two or three different positions in the aglycone.

Simply put, the pentosides of the present invention are formed when the alcohol group of the phytosterol or phytostanol reacts with the pentose to form a glycosidic bond. One preferred method of conducting this synthesis involves the creation of a pentose derivative in which the C-1 carbon of a "protected" carbohydrate is converted to a halide or halide acetate derivative. This derivative is then reacted with the phytosterol or phytostanol under suitable reaction conditions to form the pentoside. Generally, the carbohydrate is acetylated or otherwise protected in accordance with methods known in the art. The carbohydrate is converted to its acylated form to protect the sugar portion during subsequent reactions and to control the reaction products when the sugar is reacted with the sterols.

This step of protecting the carbohydrate is most preferably by a two-step esterification reaction. Any carboxylic acid anhydride can be used to make the esters. Preferably acetic acid anhydride is used to protect the hydroxyl groups. The carbohydrate is reacted with an acid anhydride in the presence of a base. Pyridine, as the solvent, works well in this reaction. The reaction can be conducted at ambient temperatures.

The partially acylated carbohydrate is then reacted with additional carboxylic acid anhydride and a catalytic amount of concentrated or anhydrous sulfuric acid. This opens the anhydro bridge and adds 2 more acyl groups. The carbohydrate group is now fully acylated. The acylated derivatives are usually crystalline and formed in good yield.

One preferred form of the present invention is shown in Figure 1, in which the carbohydrate (1) is protected at step I by reacting the carbohydrate with acetic anhydride (Ac2O) in the presence of pyridine to form compound 2, the "protected" carbohydrate. It is to be understood that the carbohydrate may be protected by the formation of any suitable acylated derivative. The C-1 of the protected carbohydrate is then converted at

step II to a halide (chloride, fluoride, bromide or iodide), indicated as compound 3. This halide is then reacted at step III with phytosterols/phytostanols (4) using an appropriate coupling agent, shown in Figure 1 as mercuric cyanide. The end product is a pentose glycoside (5) in which the carbohydrate groups remain largely protected.

Figure 2 outlines another preferred process in which the C-1 of the protected carbohydrate is then converted at steps IV and V to either a trichloroacetate or trifluroacetate derivative, indicated as compound 7, wherein R'=CX3COO(X=F, Cl). This derivative is then reacted st step VI with phytosterols/phytostanols (4), the preferred coupling agent being boron trifluroide etherate. As above, the end product is a pentose glycoside (5) in which the carbohydrate groups remain largely protected.

Figure 3 outlines the steps in converting the coupled product shown as 5 to a phytosterol glycoside 8, the final product. This involves the conversion of the protected carbohydrate groups back to the hydroxyl groups using any base such as carbonates, hydroxides, alkoxides and the like.

In a further preferred embodiment of the present invention, esters of the glycosides described herein may be prepared by methods known in the art, for example, by esterification of the free OH-groups or by trans-esterification of the acetates.

Advantages of Novel Glycoside Derivatives

The novel derivatives of the present invention afford many dietary and therapeutic advantages when compared to the use of phytosterols/phytostanols without such attachment. The most important advantage of the novel derivatives of the present invention is enhanced solubility. This is critical in the manufacture of "delivery" vehicles as described further.

Methods of Use

The pentoside derivatives of the present invention may be used directly and without further modification in cooking, baking and the like as agents to lower serum cholesterol in animals, particularly humans. They may be added to any edible oil and used for cooking, baking, and general use. Alternatively, the derivatives may be treated to enhance delivery into various other delivery media. For example, the present invention fully contemplates the formation of oleaginous gel foodstuffs such as peanut butter, mayonnaise, ice cream and margarine spreads incorporating such derivatives. There are numerous modes or "vehicles" of delivery of this composition, accordingly, this invention is not intended to be limited to the following delivery examples.

1) Pharmaceutical Dosage Forms:

It is contemplated within the scope of the present invention that the derivatives of the present invention may be incorporated into various conventional pharmaceutical preparations and dosage forms such as tablets (plain and coated) for use orally, bucally or lingually, capsules (hard and soft, gelatin, with or without additional coatings) powders, granules (including effervescent granules), pellets, microparticulates, solutions (such as micellar, syrups, elixirs and drops), lozenges, pastilles, ampuls, emulsions, microemulsions, ointments, creams, suppositories, gels, and transdermal patches, modified release dosage forms together with customary excipients and/or diluents and stabilizers.

The derivatives of the present invention, adapted into the appropriate dosage form as described above may be administered to animals, including humans, orally, by injection (intra-venously, subcutaneously, intra-peritoneally, intra-dermally or intra-muscularly), topically or in other ways. Although the precise mechanism of action is unclear, the derivatives of the present invention, administered intra-venously, lower serum cholesterol. It is believed that some blends of phytosterols, in concert, may have, in

addition to the role as an inhibitor of cholesterol absorption in the intestine, a systemic effect on cholesterol homeostasis through bile acid synthesis, enterocycte and biliary cholesterol excretion, bile acid excretion and changes in enzyme kinetics and cholesterol transport between various compartments within the body (PCT/CA97/00474 which was published on January 15, 1998).

The derivatives as described herein may be used in both dietary and therapeutic capacities in order to treat and/or prevent CVD, its underlying conditions such as hypercholesterolemia, hyperlipidemia, arteriosclerosis, hypertension, thrombosis, related diseases such as Type II diabetes, as well as other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer. In populations, which are considered "high-risk" for CVD or any of the oxidation related disorders, it is contemplated that the compositions and foodstuffs in which they are contained be used in primary, secondary and tertiary treatment programs.

In order to appreciate the various possible vehicles of the delivery of the compositions, the list below is provided. The doses of the derivatives will vary depending upon, among other factors, the mode of delivery (i.e. how and into which food or beverage or pharmaceutical the derivatives are ultimately incorporated), the patient size and condition, the result to be achieved, as well as other factors known to those skilled in the art of food additives and medicinal agents. Generally, however, it is preferred that the derivatives of the present invention be administered to humans in a form comprising up to 6 grams (based on a 70kg person) of phytosterols and/or phytostanols per day, more preferably from 1-5 grams per day and most preferably 1.5 grams per day. It will also be recognized that the provision of much larger daily doses of the derivatives are not harmful to the animal host, as excess will simply pass through normal excretory channels.

2) Foods/Beverages/Nutraceuticals:

In another form of the present invention, the derivatives of the present invention may be incorporated into foods, beverages and nutraceuticals, including, without limitation, the following:

1) Dairy Products --such as cheeses, butter, milk and other dairy beverages, spreads and dairy mixes, ice cream and yoghurt;

2) Fat-Based Products--such as margarines, spreads, mayonnaise, shortenings, cooking and frying oils and dressings;

3) Cereal-Based Products--comprising grains (for example, bread and pastas) whether these goods are cooked, baked or otherwise processed;

4) Confectioneries--such as chocolate, candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, icings and other fillings;

5) Beverages-- whether alcoholic or non-alcoholic and including colas and other soft drinks, juice drinks, dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and

6) Miscellaneous Products--including eggs and egg products, processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like.

The derivatives of the present invention may be incorporated directly and without further modification into the food, nutraceutical or beverage by techniques such as mixing, infusion, injection, blending, dispersing, emulsifying, immersion, spraying and kneading. Alternatively, the derivatives may be applied directly onto a food or into a beverage by the

consumer prior to ingestion. These are simple and economical modes of delivery.

While the following examples are intended to illustrate various aspects of the present invention and to assist in the preparation of the pentose glycosides, they are not intended to limit the scope of invention as claimed herein.

EXAMPLE 1

Synthesis of 1-0-stanoxy-2,3,4-tri-O-acetyl-B-D-xylopyranoside (with reference to Figure 1)

a) 2,3,4-tri-0-acetyl-1-bromo-D-xylopyranosylbromide (compound 3, X=Br, 6.8g) (prepared from compound 2, 1,2,3,4-tetra-O-acetyl D-xylopyranoside according to the standard method known in the art);
b) stanols (compound 4 4.1g--campestanol, R=H:36.4w/w%; sitostanol, R=CH3:62.3%w/w%;
c) mercury cyanide (5g); and
d) dry 1,2-dichloroethane (100ml)
were all added to a dry round bottom flask. The mixture was stirred at 50 °C for 24 hours. The reaction mixture was washed with water (100ml), 10% aqueous sodium iodide (2x100ml), sat, NaHCO3 (2x100ml), and water (2x100ml). The organic layer was dried over MgSO4 and concentrated. The resulting residue was purified by column chromatography to afford compound 5 (3.2g).

EXAMPLE 2

Synthesis of 1-O-trichloroaxetyl-2,3,4-tri-O-acetyl-α-D-xylopyranoside (compound 7-with reference to Figure 2)
Trichloroacetic anhydride (18ml), sodium trichloroacetate (2.5g), 2,3,4-tri-O-acetyl-D-xylopyranoside (compound 6, 5.6g and prepared from compound 2, tetra-O-acetyl xylose by hydrolysis) and anhydrous methylene chloride were added to a dry round bottom flask.

The reaction mixture was refluxed under a stream of argon until TLC showed the complete disappearance of the starting material (3 hours). The mixture was filtered through a short column of silica gel. The silica gel was washed with anhydrous methylene chloride (40x4). The combined solution was washed with water (100ml), sat NaCO3 (2x100ml) and water (100ml) respectively. The organic layer was dried over MgSO4. The solvent was evaporated to give residue (compound 7) which was used for the next step.

1-O-trichloroacetyl-2,3,4-tri-O-acetyl-D-xylopyranoside (compound 7, 8.4 g) stanols (compound 4-constituents as noted in example 1 above, 4.1g), boron trifluoride dimethyl etherate (1.3ml) and dry methylene chloride (100ml) were added to a dry round bottom flask. The reaction stirred under a stream of argon at room temperature for six hours The reaction was monitored by TLC (mobile phase: Hexanes/EtOAc=2/1). The reaction mixture was washed with water (100ml), sat NaCO3 (2x100ml) and water (100ml). The organic layer was dried over MgSO4 and concentrated. The resulting residue was purified by a chromatographic column to yield compound 5 (3,7g).

EXAMPLE 3

Synthesis of 1-0-stanoxy-2,3,4-tri-O-acetyl-D-B-xylopyranoside (compound 8--with reference to Figure 3)

1-0-Stanoxy-2,3,4-tri-O-acetyl-B-D-xylopyranoside (compound 5, 3.7g) was dissolved in ethanol (100ml) and potassium carbonate (5g) was added. The mixture was stirred at room temperature for 24 hours, filtered and washed with ethanol. The combined ethanol solution was neutralized with acetic acid and evaporated. The solid was dried under vacuum to yield 1-0-stanoxy-B-D-xylopyranoside (compound 8, 2.8g).

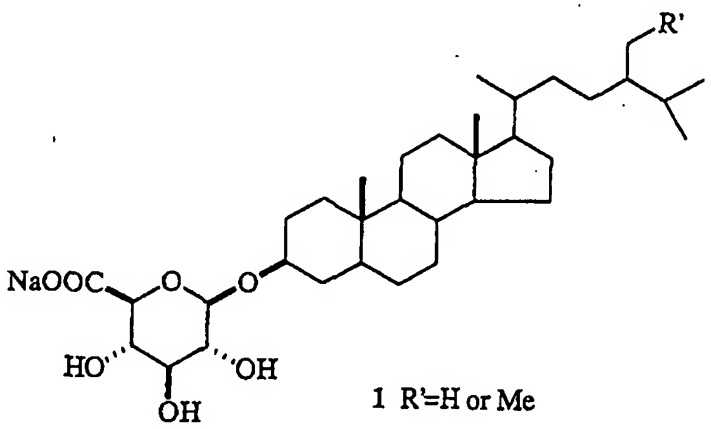
EXAMPLE 4

Synthesis of 1-0-stanoxy-B-D-xylopyranoside

1-0-Stanoxy-2,3,4-tri-O-acetyl-B-D-xylopyranoside (compound 5, 16 g) was dissolved in methanol (500ml) and sodium methoxide (0.74 g) was added. The mixture was stirred at room temperature for 24 hours. The precipitate was filtered and washed with methanol to give a crude product. The crude product was recrystallized with chloroform and methanol to afford the final product (compound 8)

EXAMPLE 5

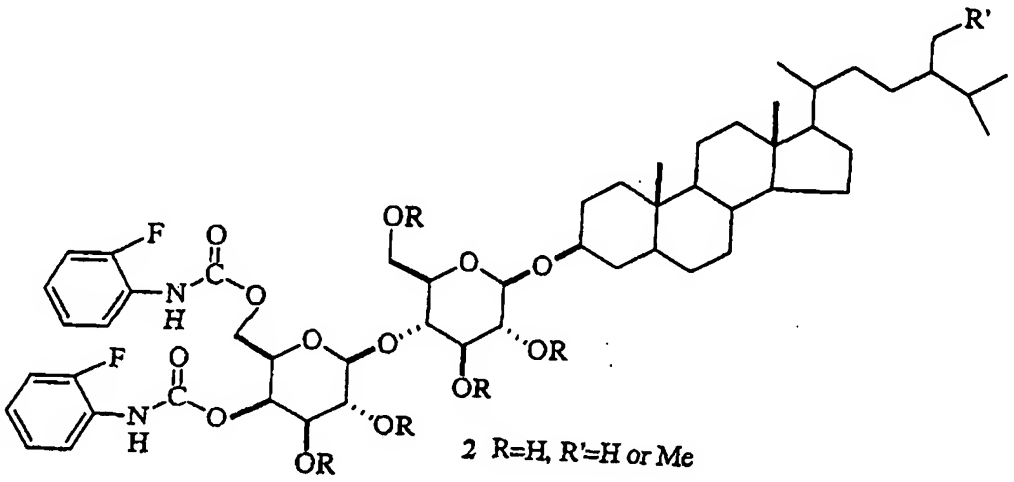
The following product, 1-0-phytostanol-B-D-glucuronic acid, and its salt were synthesized in accordance with the protocols noted herein and are part of the invention as claimed:



1 R'=H or Me

EXAMPLE 6

The following product, 1-0-phytostanol-4-O-[[bis(2-fluorophenyl)carbamoyl]-B-D-galactopyranosyl]-B-D-glucopyranoside, and its salt were synthesized in accordance with the protocols noted herein and are part of the invention as claimed:



2 R=H, R'=H or Me

REFERENCES

1. Law M.R., Wald N.J., Wu,., Hacksaw ZA., Bailey A.; Systemic underestimation of association between serum cholesterol concentration and ischemic heart disease in observational studies: Data from BUPA Study; *Br. Med. J.* 1994; 308:363-366

2. Law M.R., Wald N.J., Thompson S.G.; By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease? *Br. Med. J.* 1994; 308:367-373

3. La Rosa J.C., Hunninghake D.. Bush D. et al.; The cholesterol facts: A summary of the evidence relating to dietary fats, serum cholesterol and coronary heart disease:Ajoint statement by the American Heart Association and the National Heart, Lung and Blood Institute. *Circulation* 1990; 81:1721-1733

4. Havel R.J., Rapaport E.. Drug Therapy: Management of Primary Hyperlipidemia. *New England Journal of Medicine*, 1995; 332:1491-1498

5. Kuccodkar et al.; Effects of plant sterols on cholesterol metabolism. *Atherosclerosis*, 1976; 23:239-248

6. Lees R.S., Lees A.M. Effects of sitosterol therapy on plasma lipid and lipoprotein concentrations. In: Greten H (Ed) Lipoprotein Metabolism. Springer-Verlag, Berlin, Heidelberg, New York, 1976:119-124

7. Lees A.M., Mok H.Y.I., Lees R.S., McCluskey M.A., Grundy S.M. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atherosclerosis* 1977; 28: 325-338

WE CLAIM:

1. A novel glycoside compound comprising a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof.

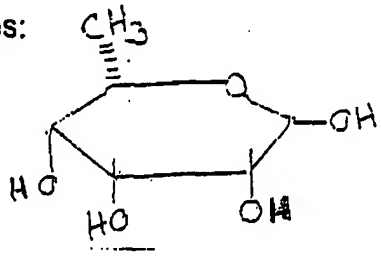
2. The compound of claim 1 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

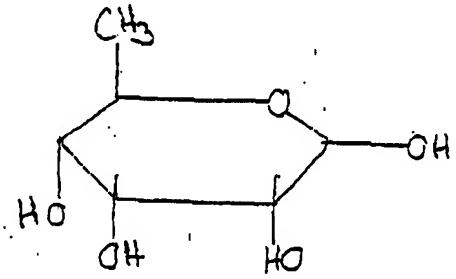
3. The compound of claim 1 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers.

4. The compound of claim 1 wherein the pentose is selected from the group consisting of D-ribose, D-arabinose, D-xylose, D-lyxose, L-ribose, L-arabinose, L-xylose, L-lyxose, D-ribulose, L- ribulose, D-xylulose and L-xylulose.

5. The compound of claim 1 wherein the pentose is a methyl pentose selected from the group consisting of L-rhamnose, L-isorhamnose, D-isorhamnose, fucose, rhodeose, and epirhodeose.

6.The compound of claim1 wherein the pentose is a methyl pentose having one of the following two structures:





7. A method of treating or preventing cardiovascular disease and its underlying conditions including athereosclerosis and hypocholeserolemia in an animal which comprises administering to the animal a novel glycoside compound comprising a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof.

8. The method of claim 7 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

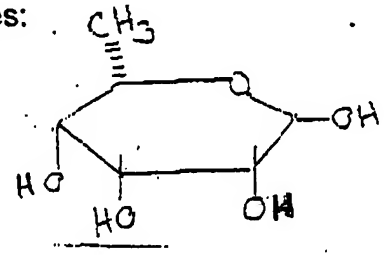
9. The method of claim 7 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers.

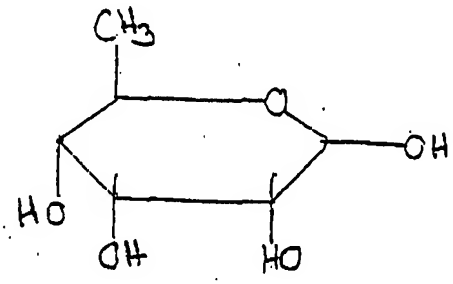
10. The method of claim 7 wherein the pentose is selected from the group consisting of

D-ribose, D-arabinose, D-xylose, D-lyxose, L-ribose, L-arabinose, L-xylose, L-lyxose, D-ribulose, L- ribulose, D-xylulose and L-xylulose.

11. The method of claim 7 wherein the pentose is a methyl pentose selected from the group consisting of L-rhamnose, L-isorhamnose, D-isorhamnose, fucose, rhodeose, and epirhodeose.

12. The method of claim 7 wherein the pentose is a methyl pentose having one of the following two structures:





13. The method of claim 7 wherein the animal is human.

14. A method of lowering serum cholesterol is an animal which comprises administering to the animal a novel glycoside compound comprising a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof.

15. A product therapeutically effective at treating and preventing cardiovascular disease and, in particular its' underlying conditions atherosclerosis and hypercholesterolemia which comprises a novel glycoside compound comprising a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof and a pharmaceutically acceptable or

food-grade carrier therefore.

16 A comestible or beverage comprising a novel glycoside compound comprising a carbohydrate moiety and a non-carbohydrate moiety wherein the carbohydrate moiety is selected from the group consisting of pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives and the non-carbohydrate moiety is either a phytosterol, a phytostanol or a derivative thereof.

17. A process of preparing glycosides having a carbohydrate moiety and a phytosterol or phytostanol moiety, which comprises:
a) protecting the carbohydrate moiety;
b) converting the protected carbohydrate moiety to a halide or halide/acetate derivative; and then
(c) adding phytosterol or phytostanol to the derivative under reaction conditions suitable to form a glycoside.

18. The process of claim 17 wherein the carbohydrate moiety is selected from the group comprising pentose monosaccarides, disaccharides, trisaccharides and oligosaccharides and their acylated derivatives.

19. The process of claim 17 wherein the halide with which the protected carbohydrate moiety is reacted at step b is selected from the group consisting of fluoride, bromide, iodide, and chloride.

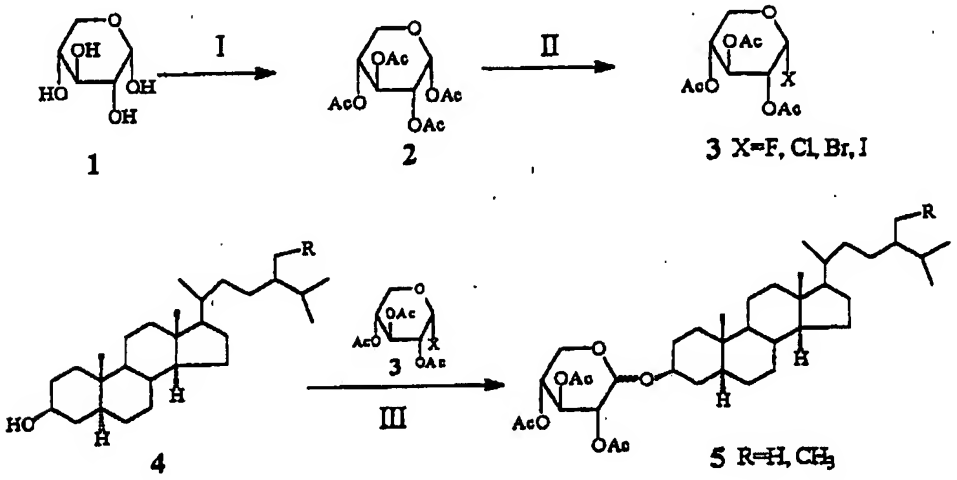
20. The process of claim 17 wherein the halide/acetate derivative with which the protected carbohydrate moiety is reacted at step b has the formula CX3COOH and wherein X is selected from the halogens.

21. The process of claim 17 additionally comprising step d) in which a basic reagent is

reacted with the glycoside so formed in order to covert the protected carbohydrate moiety to a non-protected form.

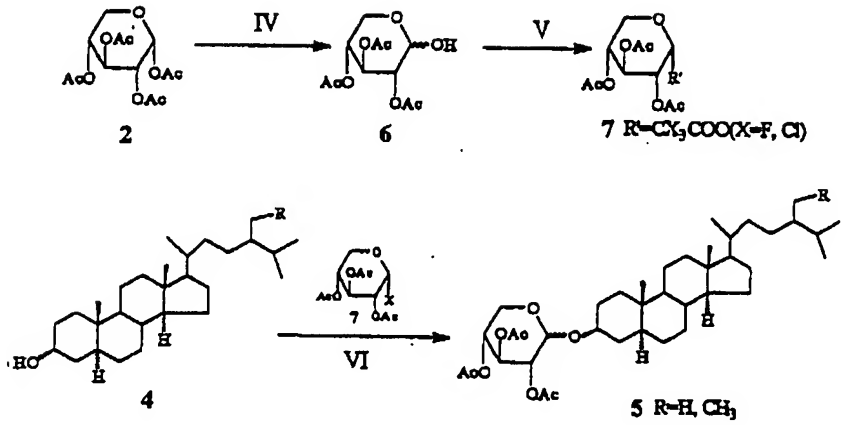
22. The process of claim 21 wherein the basic reagents are selected from the group consisting of alkali carbonates and alkali hydroxides.

Figure 1



I. Ac_2O, Pyridine;
II. HX (X=F, Br, Cl, I), 0-5°C;
III. $Hg(CN)_2$, $ClCH_2CH_2Cl$;

Figure 2



IV. THF, MeOH, NH_3;
V. $(CX_3CO)_2O$, CX_3COONa, CH_2Cl_2 (X=F, Cl);
VI. $BF_3 \cdot Et_2O$, CH_2Cl_2;